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~~DESCRIPTION~~

RHEUMATOID ARTHRITIS GENE AND METHOD FOR DIAGNOSING RHEUMATOID ARTHRITIS

BACKGROUND OF THE INVENTION

~~Technical~~ 1. Field of the Invention

The present invention relates to the disease gene of rheumatoid arthritis present in the human X chromosome and a method for diagnosing rheumatoid arthritis by detecting the presence of the disease gene or its expression product.

2. Description of the Related ~~Background~~ Art

Although aspects, particularly the pathological process, of arthritis and arthritis mutilans which cause rheumatoid arthritis, have been clarified through various investigations, because most autoimmune diseases associated with rheumatoid arthritis developed or worsen into the disease only when various causative factors coincide, the interaction itself of multiple factors must be clarified to understand the disease and to develop appropriate methods of treatment.

The number of patients with rheumatoid arthritis in the world is 1% or less (N. Engl. J. Med. 322: 1277-1289, 1990), but among sibilings of patients, over 8% develop the disease (Cell. 85: 311-318, 1996), which leads to the notion that some genetic factor may be involved. However, molecular genetic

(3) A disease gene of rheumatoid arthritis located within ±1 centi Morgan vicinity of a DNA sequence on human chromosome X to which microsatellite marker(s) DXS1001, DXS1047, DXS1205, DXS1227 and/or DXS1232 hybridize(s).

The present inventors identified, as a result of further studies on each of the rheumatoid arthritis genes specified in the above-described previous application, the specific gene regarding the disease gene (3) described above and determined its molecular structure.

Summary of Invention

In order to solve the above-described problems, the present invention provides a cDNA of a disease gene for rheumatoid arthritis, ~~which is a mutant of protooncogene Dbl transcribing an mRNA that encodes the cDNA of~~ which has the sequence ~~from the 2679th to 2952nd bases is shown in~~ of SEQ ID NO: 1, ~~which disease gene transcribes an mRNA encoding the cDNA of which the region from the 20th to 274th bases in SEQ ID NO: 1 is substituted with the sequence of SEQ ID NO: 2~~.

The present invention also provides ~~a cDNA of the above disease gene,~~ a DNA fragment, which is a part of such cDNA and preferably contains the base sequence from 2693rd to 2702nd of SEQ ID No: 1, protein expressed by the above disease gene, a peptide which is a part of such protein, and an antibody against such protein.

Further, the present invention provides a method for diagnosing rheumatoid arthritis comprising the detection of the

Figure 1 shows the base sequence of the 2679th to 2952nd bases of the Dbl gene cDNA in its normal form (see bases 2679 to 2952 of SEQ ID No:5), the corresponding base sequence of the cDNA of the disease gene of RA (see bases 2679 to 2757 of SEQ ID No:1), and the respective amino acid sequence (1 letter notation) encoded by these sequences (see amino acid residues 836 to 860 of SEQ ID NO:2 and amino acid residues 836 to 925 of SEQ ID No:6)

mRNA from the above disease gene or the above protein in a biological specimen.

The present invention further provides a method for the functionally complementing Dbl deficiency.

Brief Description of the Drawing

Description of the Preferred Embodiments

~~Best Modes for Carrying Out the Invention~~

Hereinafter, embodiments of the present invention having the above-described characteristics will be described.

The ~~rheumatoid arthritis disease gene~~ cDNA of the disease gene for rheumatoid arthritis of the present invention (hereinafter referred to as "~~RA disease gene~~ cDNA") is a variant sequence of known protooncogene Dbl ~~gene~~ cDNA (EMBO J. 7(8): 2463-2473, 1988; GenBank Accession No. X12556) (SEQ ID No:5) ~~which is isolated from human chromosome X by the method described in the after-mentioned Examples. In other words, this Dbl gene transcribes the mRNA encoding the cDNA for which the sequence of the 2679th to 2952nd bases is represented in SEQ ID NO: 1, while in the cDNA of the variant gene, the sequence of the 3' side of the 241st base in SEQ ID NO: 1~~ In the cDNA of the present invention, 2919th base in GenBank/X12556 (SEQ ID NO:5) is linked to the downstream side of the ~~18th~~ 2696th base in GenBank/X12556 (SEQ ID NO:5) to induce a frame shift in amino acid translation, ~~causing the 19th to 274th base in SEQ ID NO: 1 to be substituted by the sequence shown in SEQ ID NO: 2~~ (and eliminate bases 2697 to 2919 of SEQ ID NO:5) creating the sequence shown in SEQ ID NO:6. Fig. 1 shows the base sequence of the 2679th to 2952nd bases (~~same as SEQ ID NO: 1~~) of the Dbl gene cDNA in its normal form (see bases 2679 to 2952 of SEQ ID No:5), the corresponding base sequence of the cDNA of the ~~RA~~ disease gene of RA (see bases 2679 to 2757 of SEQ ID NO:1), and the respective amino acid sequences (1 letter notation) encoded by these sequences (See amino acid residues 836 to 860 of SEQ ID NO:2 and amino acid residues 836 to 925 of SEQ ID NO:6).

In addition, generally, polymorphism of individual differences is often found for human genes. Thus, the RA disease gene of the present invention may include genes that code cDNAs obtained by the addition, deletion or substitution of one or more nucleotide in SEQ ID NO: 2. Likewise, the present invention also includes proteins with one or more amino acid added to, deleted from and/or substituted, produced by such change to the base.

The cDNAs of the present invention may easily be isolated by, for example, the method described in the after-mentioned Example. Further, the cDNAs of the present invention may be cloned from a cDNA library produced by a known method (Mol. Cell. Biol. 2:161-170, 1982; J. Gene 25: 263-269, 1983; Gene 150: 243-250, 1994) using poly(A)+RNA extracted from cells of a patient with rheumatoid arthritis. Such cloning may be performed by, for example, synthesizing oligonucleotides based on the sequence information provided by the present invention and screening by colony or plaque hybridization by a known method using the resultant oligonucleotides as probes. Also, oligonucleotides, which hybridize to both ends of the target cDNA fragment, may be synthesized, and using them as primers, the cDNA of the present invention may be produced by RT-PCR method from mRNAs isolated from cells of a patient with rheumatoid arthritis.

The DNA fragment of the present invention comprises a

portion of the aforesaid cDNA, and necessarily contains the base sequence from 2693rd to 2702nd of SEQ ID No: 1. shown in ~~SEQ ID NO: 3~~. In other words, ~~SEQ ID NO: 3~~ is the underlined sequence in Figure 1, and is a characteristic region, which is not present in normal Dbl gene or its cDNAs. Further, the DNA fragment includes both sense and antisense strands. These DNA fragments may be used as probes for genetic diagnosis.

The proteins of the present invention are expression products resulting from the RA disease genes of the present invention, and has the amino acid sequence shown in SEQ ID NO: 2 ~~at its C-terminal~~. These proteins may be obtained by chemical peptide synthesis method based on the amino acid sequence provided by the present application, or by recombinant DNA technique using cDNAs provided by the present application. For example, when recombinant DNA technique is used to obtain the proteins, RNA may be prepared by *in vitro* transcription using a vector containing the cDNA of the present invention; using this RNA as a template, the proteins may be obtained by *in vitro* translation. Also, the coding region of the cDNA may be recombined into an appropriate expression vector by any known method, and the recombinant vector obtained may be used to transform *E. coli.*, *Bacillus subtilis*, yeast, animal cells or the like, whereby expression of the protein in bulk would be possible using these recombinant cells.

When *in vitro* translation is used to produce the proteins of the present invention, the coding region of the cDNA of the

analysis, and the maximum Lod value was calculated by single point analysis.

As a result, the maximum Lod was determined to be 2.03 for DXS984, which is located in the 0.1 centi Morgan vicinity of DXS1232, one of the candidate genetic loci disclosed by the present inventors (International Immunology 10(12): 1891-1895; Journal of Clinical Rheumatology 4(3): 156-158, 1998), showing significant correlation. By searching the international data base on the internet (Genemap98, http://www.ncbi.nlm.nih.gov/genemap98/), it was found that the physical location of DXS984 was 4259 cR10000(F) on the G3 Radiation hybrid map, and thus it was proved that the protooncogene Dbl was situated nearest to DXS984.

<Example 2> Analysis of Abnormal Dbl Gene

In order to compare the cDNAs between Dbl genes, cDNA was synthesized by reverse transcription using RT-PCR kit (Perkin Elmer Inc.) from the total RNA obtained from peripheral blood of RA disease patients collected using Isogen agent (Nippongene Co. Ltd.), and dissolved in 20μl of sterilized water. Furthermore, primers (SEQ ID NO: 3 and 4) were prepared using the Dbl cDNA sequence (Genbank Accession No. X12556) (Amersham Pharmacia), and part of the Dbl cDNA sequence was isolated by the PCR method. The composition of the reaction solution for PCR was: 10 pmol each of forward primer (SEQ ID NO: 3) and reverse primer (SEQ ID NO: 4), approximately 0.1μg of template DNA, 2.5μl

of LA-PCR buffer (Takara Shuzo Co. Ltd.), 4.0μl of 2.5mM dNTP Mix, 0.25μl of LA Taq enzyme (Takara Shuzo Co. Ltd.) and 2.5μl of 25mM $MgCl_2$ mixed, after which sterilized water was added to obtain a total volume of 25μl. The reaction was performed in a thermal cycler (PTC-200) of MJ Research by repeating 35 cycles of the process of heat denaturation at 94°C for 30 seconds, primer annealing at 52°C for 30 seconds and extension at 72°C for 2 minutes. The PCR products were subjected to electrophoresis of conventional methods, in TAE buffer solution using 1% Agarose L (Nippongene Co. Ltd.) gel and DNA molecular weight markers (200bp ladder) by Promega Co., to confirm the amplified bands. As a result, it was found that the size of normal DNA was 660bp while the size of DNA chain from some patients were distinctly shorter (approximately 440bp).

Next, after each respective bands were cut out, the gels were melted at 65°C for 10 minutes, and the DNAs were purified by conventional phenol extraction methods and ethanol precipitation methods. Then, using 100ng of the resultant DNA as a template, cycle sequence reaction and purification were performed following the specifications of the manufacturer of BigDye terminator cycle sequence kit by Perkin Elmer Inc., and the sequence was determined by a Type AB1377 DNA sequencer of Perkin Elmer Inc. As a result, it was evident that in the above-described abnormally short DNA, as shown in Fig. 1, the 223bp from the number 2697 to number 2919 bases are deleted,

making it 437 bp. This result indicates that with the amino acid deletion encoded in the genetic information downstream of base number 2693, and by inducing frame shift, abnormal polypeptide chain short of 65 amino acids is produced.

~~Industrial Applicability~~

As described in detail above, the present invention provides a cDNA of a disease gene for rheumatoid arthritis occurring in human chromosome X. This invention enables the easy and reliable diagnosis of rheumatoid arthritis. Furthermore, this invention is useful for the development of novel treatment and therapeutic agents for rheumatoid arthritis.

ABSTRACT OF THE DISCLOSURE

As a disease gene for rheumatoid arthritis present in human chromosome X and a method of diagnosing rheumatoid arthritis, a disease gene, which is a mutant of protooncogene Dbl transcribing an mRNA that encodes the cDNA of which the sequence from the 2679th to 2952nd bases is shown in SEQ ID NO: 1, which disease gene transcribes an mRNA encoding the cDNA of which the region from the 20th to 274th bases in SEQ ID NO: 1 is substituted with the sequence of SEQ ID NO: 2, and a method for diagnosing rheumatoid arthritis by detecting the mRNA of the above-described gene or its expression product in a biological specimen, is provided.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows:

Above claim 1, the following sentence was added:

--What is claimed is:--.

1. (Amended) A cDNA of a disease gene for rheumatoid arthritis, which ~~is a mutant of protooncogene Dbl transcribing an mRNA that encodes the cDNA of which the sequence from the 2679th to 2952nd bases is shown in SEQ ID NO: 1, which disease gene transcribes an mRNA encoding the cDNA of which the region from the 20th to 274th bases in SEQ ID NO: 1 is substituted with the~~ comprises the base sequence of SEQ ID NO: ~~2~~1.

3. (Twice Amended) A DNA fragment~~,~~ which is comprises the base sequence of a part of ~~the disease gene of claim~~SEQ ID NO: 1, and necessarily contains the base sequence from 2693rd to 2702nd of SEQ ID NO: ~~3~~1.~~-~~

7. (Twice Amended) A method for diagnosing rheumatoid arthritis, said method comprising ~~the detection of the~~detecting an mRNA from the ~~disease gene~~cDNA of claim 1, in a biological specimen.

11. (Amended) A method for diagnosing rheumatoid arthritis, said method comprising ~~the detection of the mRNA from~~detecting the protein of claim 4, in a biological specimen.